

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Patent Application No. 10/585,566

Confirmation No. 2649

Applicant: Moschel et al.

Filed: August 29, 2006

TC/AU: 1624

Examiner: Jaisle, Cecilia M.

Docket No.: 253443 (Client Reference No. E-274-2003/0-US-03)

Customer No.: 45733

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**DECLARATION UNDER 37 C.F.R. 1.132 FROM ANTHONY E. PEGG, PH.D.**

Sir:

I, Anthony E. Pegg, hereby declare that:

1. I am a co-inventor of the subject patent application. I am familiar with the patent application and pending claims.

2. A copy of my Curriculum Vitae, including my educational qualifications and technical experience, is attached hereto.

3. Based on tests carried out under my direction or supervision, *O*<sup>4</sup>-benzylfolate ("BF") reduces AGT activity in KB tumor xenografts in mice, as discussed below.

4. To reduce the concentration of free folic acid in serum to the level comparable to those observed in humans, athymic mice were kept on folate-free diet for two weeks. 10<sup>6</sup> KB cells were then injected bilaterally into the hind thighs. Tumors were allowed to grow for two weeks, before a BF solution in PBS was injected i.p. and the animals were sacrificed at different times.

5. Figure 1 shows a dose-response curve of AGT activity vs. dose of BF in tumor xenografts 8 hours after i.p. injection in mice. Figure 2 shows the time-course study of AGT

activity as a function of time at a BF dose of 150 mg/kg and 200 mg/kg i.p. BF decreased AGT activity in KB tumor tissue in a dose-dependant manner with 80% of reduction in 2 h and 90% in 4 h after the administration of 150mg/kg of the drug. AGT activity remained at this low level until 12 h after the injection. Gradual recovery of AGT activity in tumor tissues was observed at 16 and 20 h reaching 80% of control values by 24 h. These results show that administration of a second dose of BF 10 h after the fist dose would provide 90% loss of AGT activity during the interval between 4 h and 22 h. This interval should be sufficient for enhancing the cytotoxic effects of BCNU during which DNA adducts undergo chemical rearrangement and form interstrand crosslinks.

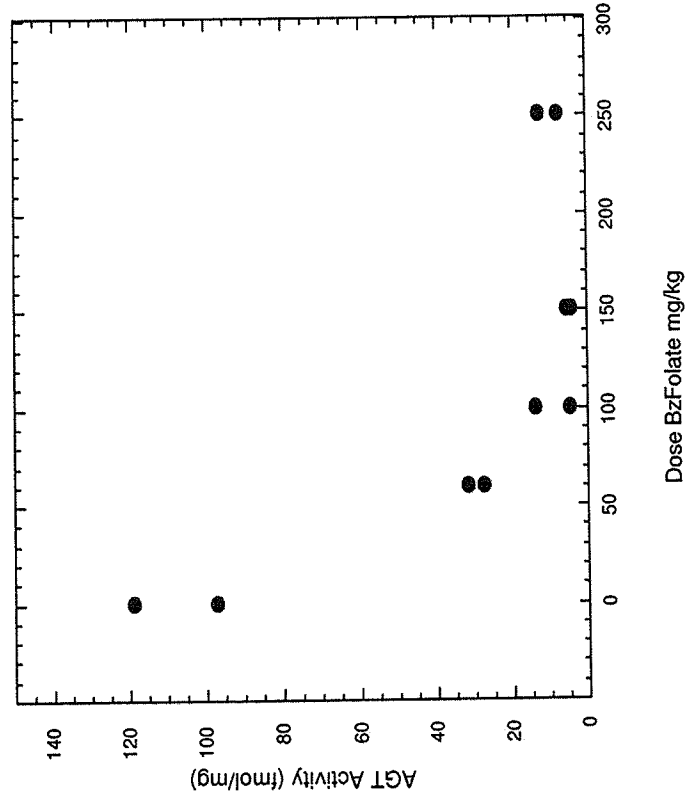
6. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Oct 27 2002  
Date

Anthony E. Pegg  
Anthony E. Pegg, Ph.D.

**FIG. 1**

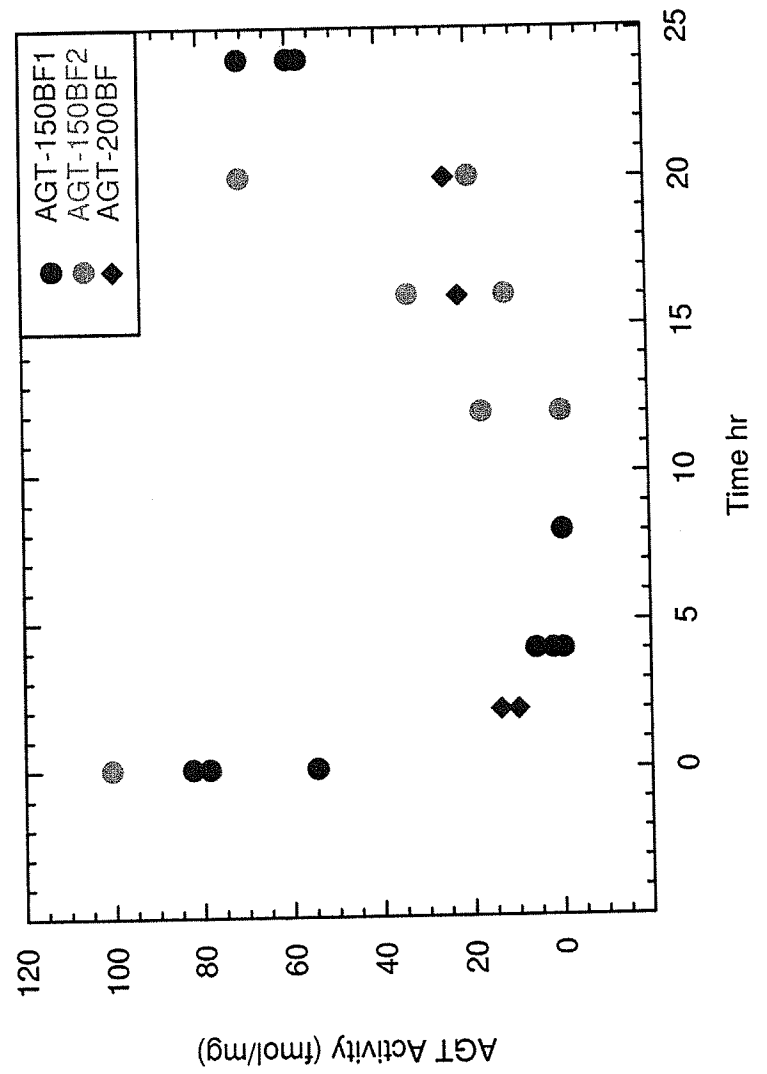
Inactivation of AGT by BenzylFolate in tumor xenografts  
(8 hrs after i.p. injection)



Dose (mg/kg)	AGT Activity (fmol/mg)
PBS	98
60	28
100	14
150	5
250	13

**FIG. 2**

Inactivation of AGT by BenzylFolate in tumor xenografts



Dose (mg/kg)	AGT Activity (fmol/mg)	
PBS	55	101
	79	
	83	
2 hr		10
		14
4 hr	0	
	2	
	6	
8 hr	0	
	0	
12 hr	0	
	18	
16 hr	12	
	34	
20 hr	20	
	71	
24 hr	58	
	60	
	71	